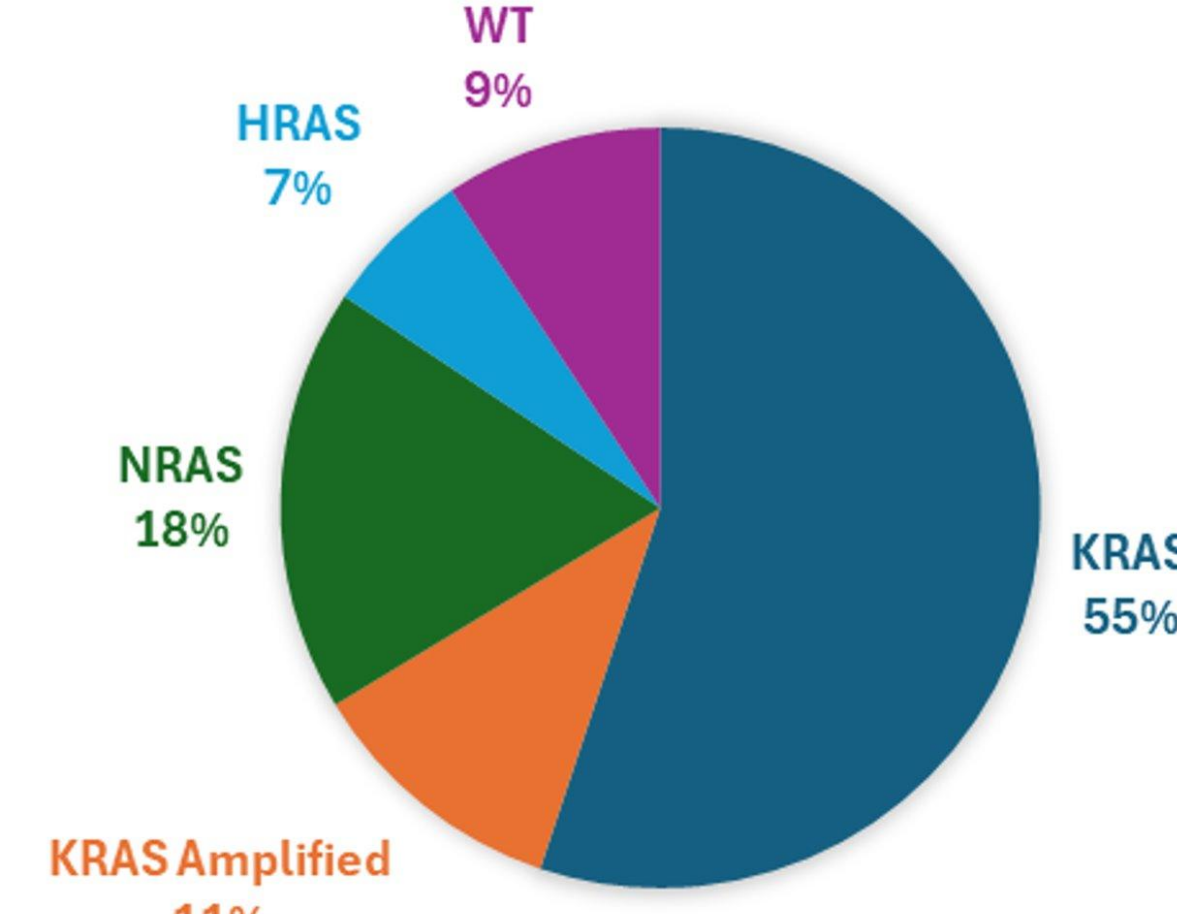


1 Introduction

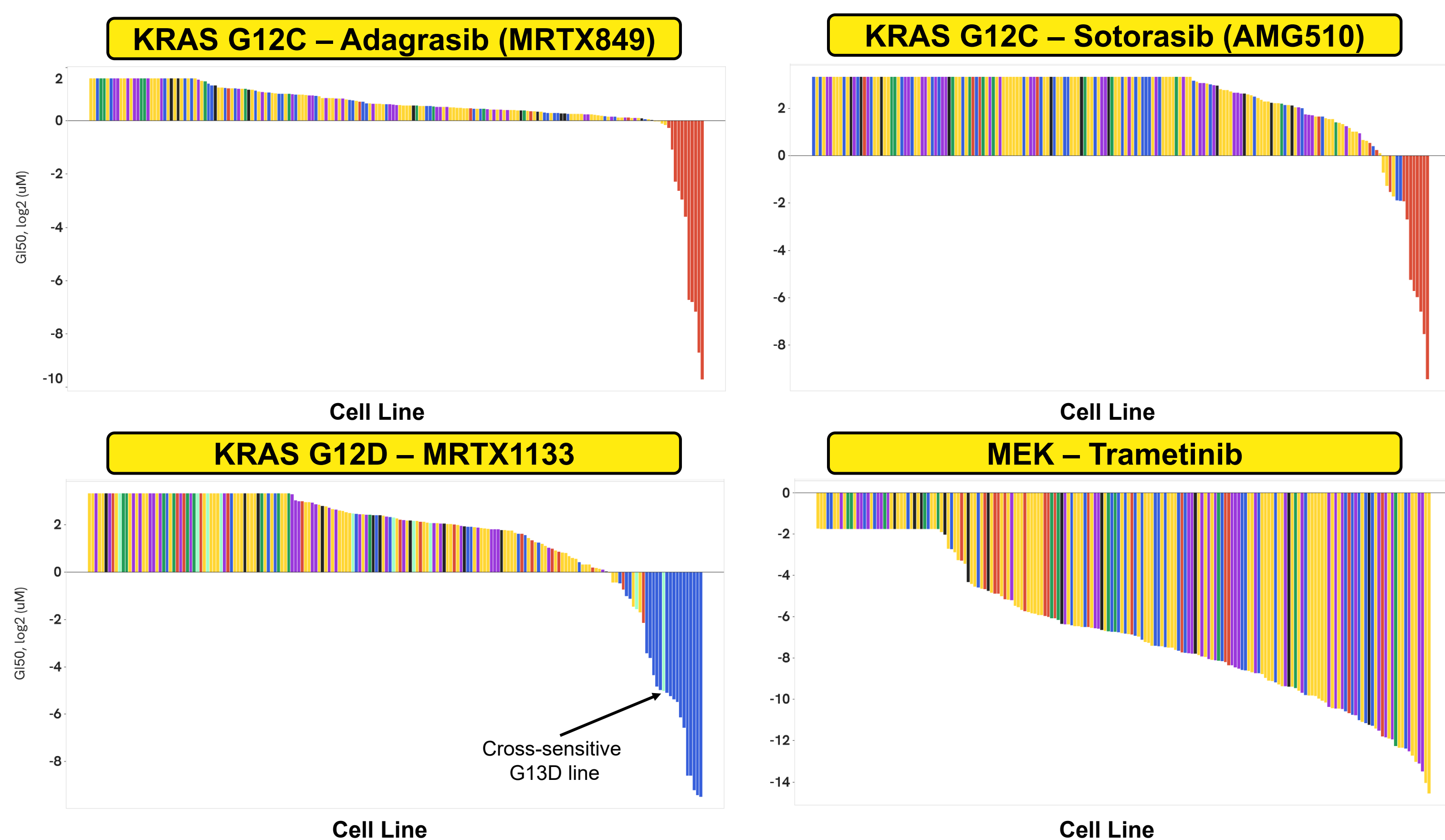
- Targeted drug profiling using cancer cell line panels is a powerful approach for evaluating efficacy, selectivity, and mechanism of action of emerging therapeutics.
- Revvity's curated RAS panel of ~200 cancer cell lines reflects the disease-relevant distribution of KRAS, NRAS, and HRAS alterations across major cancer types.
- This panel enables comprehensive interrogation of RAS pathway-targeting agents, with ~65% of the lines amenable to 3D spheroid screening, enhancing physiological relevance.
- Here, we demonstrate the platform's utility in characterizing selectivity profiles and mechanistic engagement of mutant-allele selective KRAS inhibitors.
- The RAS-focused panel facilitates nuanced assessment of compound activity across diverse genetic backgrounds, supporting identification of optimal therapeutic candidates and patient stratification.
- This approach provides a robust framework for precision oncology strategies targeting RAS-driven malignancies.



Distribution of RAS alterations across curated panel of cell lines enriched for key RAS pathway alterations and relevant tissue contexts.

2 2D RAS Cell Panel Screening Enables Mutation-Specific Characterization of Emerging RAS-targeted therapeutics

● G12C ● G12D ● G13D ● Other KRAS mutation ● HRAS Mutant ● NRAS Mutant ● Wild Type

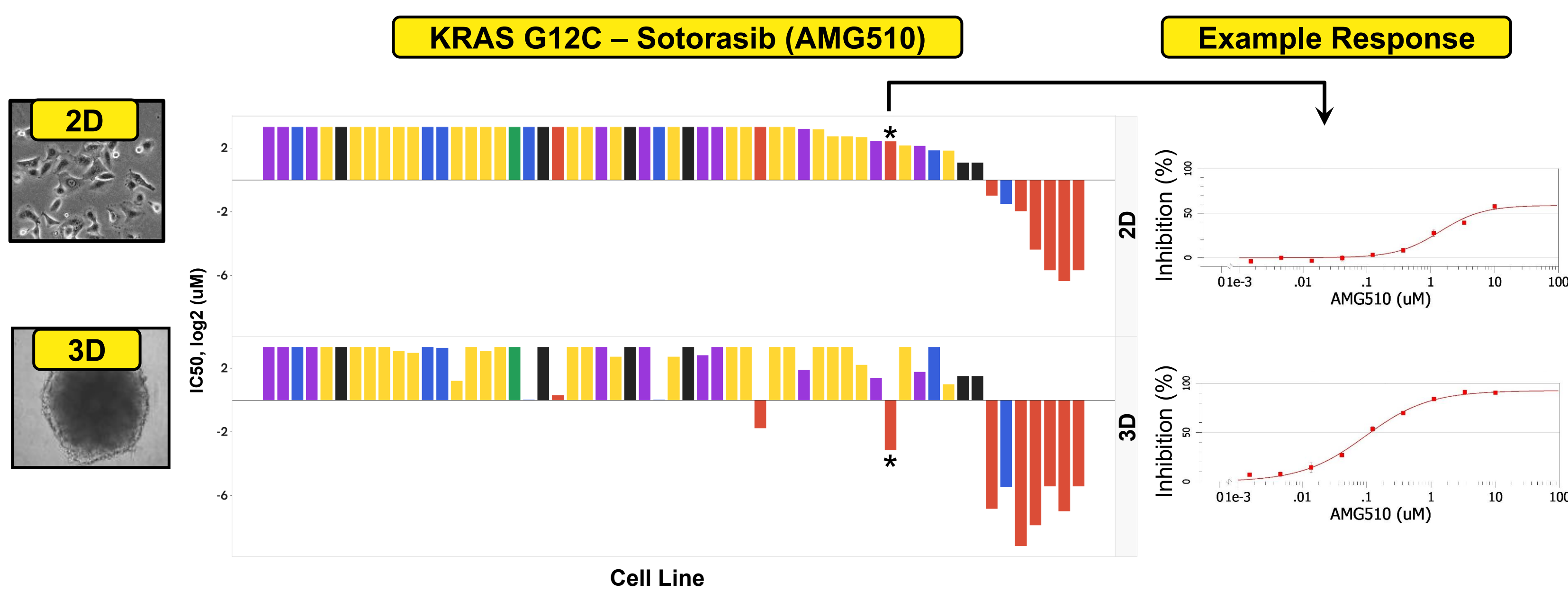


Growth inhibition (GI50) was determined in 5-day ATP-based luminescence based 2D proliferation assays across the Revvity RAS cell panel. Waterfall plots show rank-ordered sensitivity.

- G12C inhibitors (MRTX849, AMG510):** Clear selectivity for G12C-mutant cell lines, confirming reported specificity
- G12D inhibitors (MRTX1133):** Primary activity in G12D lines as expected; interestingly showed comparative activity in one G13D line hinting at cross-activity
- MEK inhibitor control (Trametinib):** Broad activity across all RAS mutations, demonstrating lack of allele selectivity

3 3D Screening Reveals Additional Insights into KRAS Dependency and Drug Activity

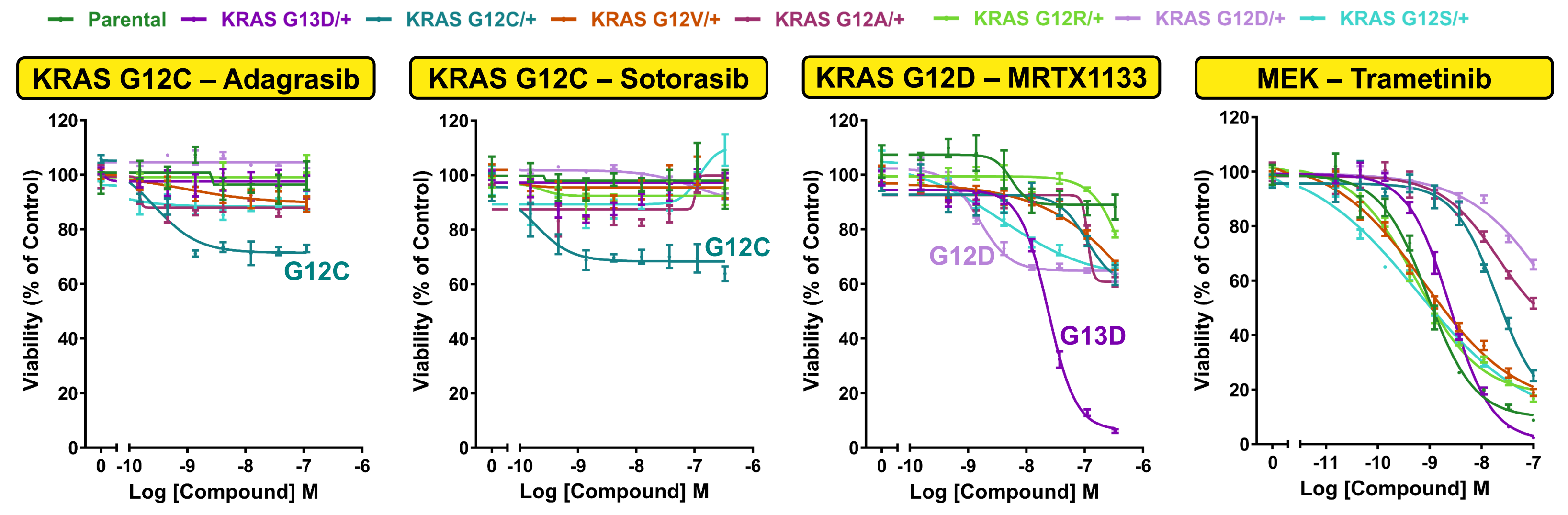
● G12C ● G12D ● Other KRAS mutation ● HRAS Mutant ● NRAS Mutant ● Wild Type



2D versus 3D responses in a subset of the KRAS panel, with cell lines rank-ordered by 2D sensitivity. 3D responses (IC50) were determined using 5-day spheroid growth assays with an ATP-based luminescence readout.

- Enhanced G12C Sensitivity:** AMG510 example shows several G12C lines with minimal 2D responses demonstrated marked 3D sensitivity
- Increased dynamic range:** G12C lines already sensitive in 2D demonstrated even greater 3D activity
- Enhanced KRAS dependency:** Increased inhibitor sensitivity in 3D is consistent with the known greater KRAS pathway dependence under 3D culture conditions
- Complementary approach:** This example demonstrates how 3D screening provides additional insights for spheroid-forming lines, creating a richer dataset with potential for enhanced clinical translatability

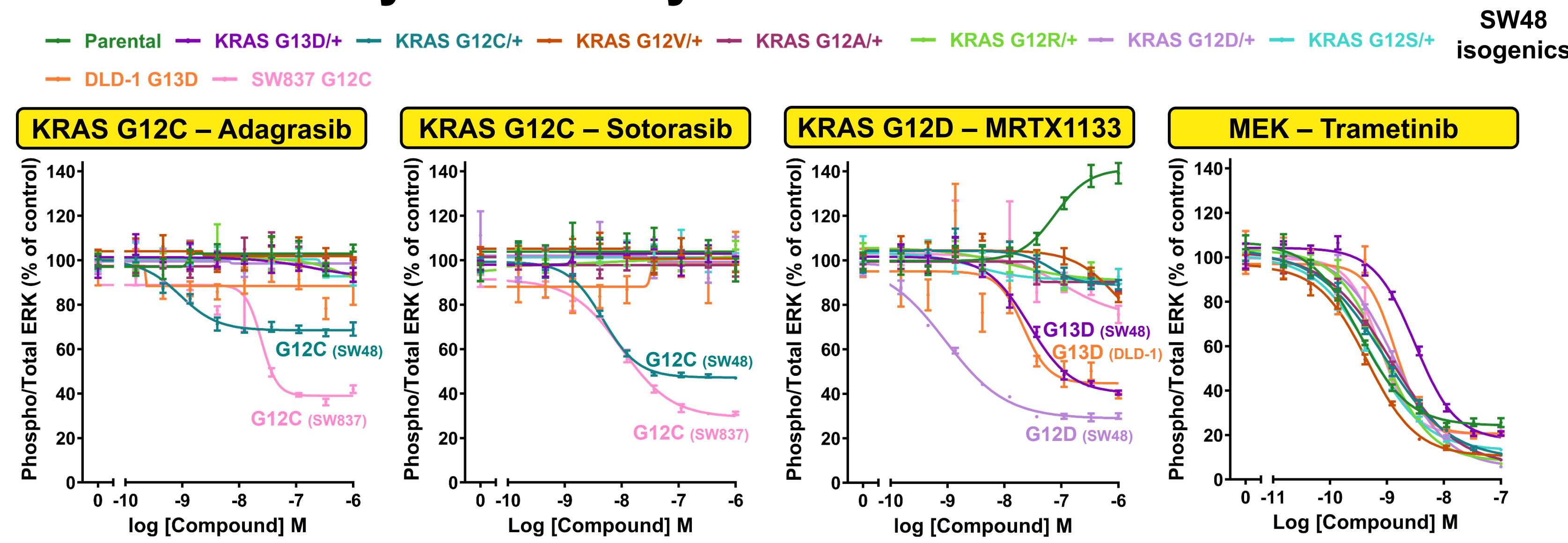
4 Isogenic Cell Panel Screening Provides Robust Validation of Mutation-Specific Selectivity for RAS Therapeutics



Viability was determined using 5-day ATPlite™ 1glowF luminescent 2D proliferation assays across Revvity's KRAS mutant isogenic panel of SW48 cells containing activating mutations of KRAS. Revvity Cell Line Catalogue No: G12A/+ (HD 103-009); G12C/+ (HD 103-006); G12D/+ (HD 103-11); G12R/+ (HD 103-010); G12S/+ (HD 103-013); G12V/+ (HD 103-007); G13D/+ (HD 103-002)

- G12C inhibitor validation:** MRTX849 and AMG510 showed activity only in G12C isogenic line, confirming specificity
- G12D inhibitor cross reactivity confirmed:** MRTX1133 was active in both G12D and G13D isogenic lines, confirming cross-reactivity observed in wider RAS panel

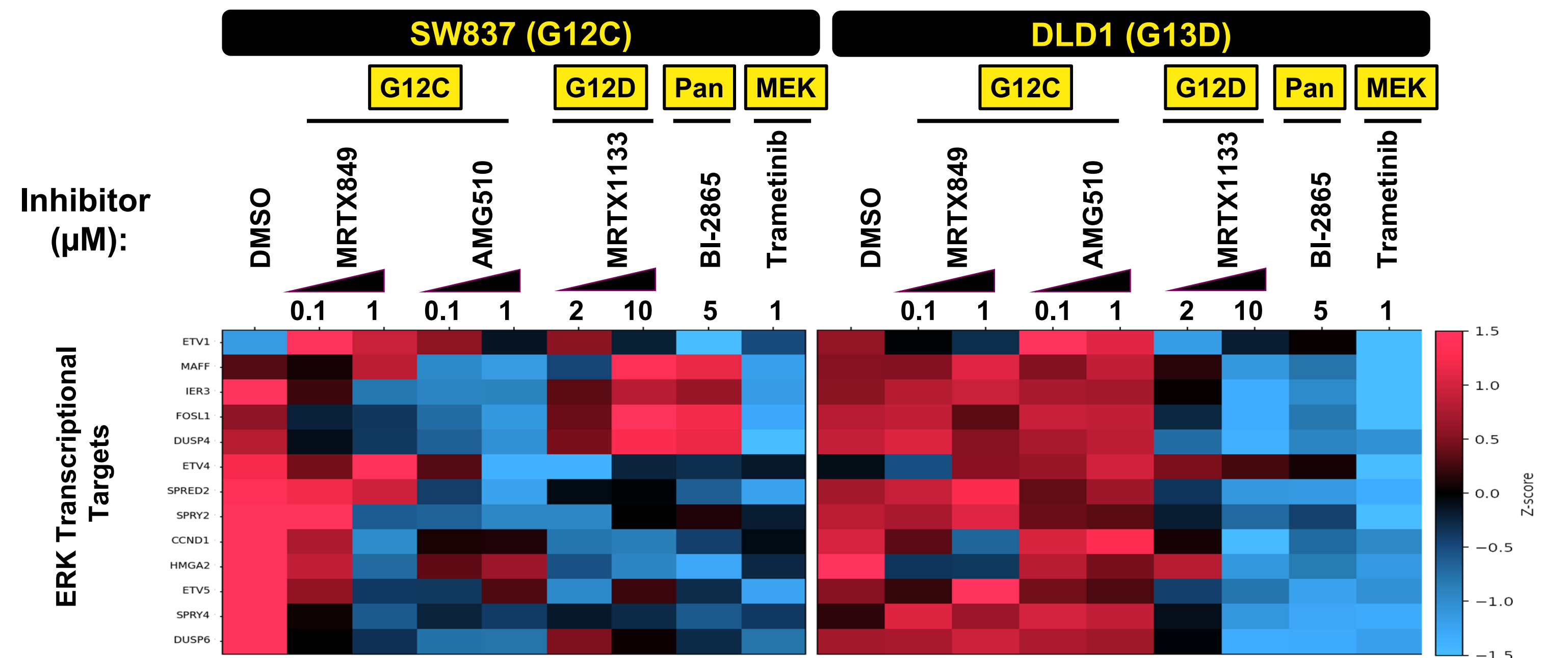
5 pERK Analysis Supports Mechanistic Understanding and Mutation-Aligned ERK Pathway Selectivity



pERK after 4h drug treatment in KRAS mutant isogenic (SW48) and non-isogenic lines in 2D measured by HTRF. pERK (Revvity; 64AERPEH) was normalized to total ERK (Revvity; 64NRKPEH).

- G12C pathway inhibition:** MRTX849 and AMG510 selectively inhibited pERK only in G12C lines, confirming selectivity observed in proliferations assays
- G12D/G13D cross-activity confirmed:** MRTX1133 inhibited pERK in the G12D isogenic line and both G13D lines, supporting cross-activity hypothesis

6 DrugSeq Transcriptomic Analysis Confirms Mutation-Aligned ERK Pathway Selectivity



ERK pathway profile following 4h treatment in 2D. DrugSeq was performed by Alithea Genomics.

- G12D/G13D cross-activity confirmed:** MRTX1133 inhibited ERK pathway activity in G13D DLD-1 and interestingly in G12C SW837, supporting cross-activity hypothesis with selectivity window for G12C hypothesized at lower doses based on the pERK analysis above (section 6)
- G12C selectively confirmed:** MRTX849 and AMG510 inhibited ERK pathway in G12C SW837 but not G13D DLD-1, confirming selectivity from previous assays

7 Conclusions

- Revvity's curated RAS panel was successfully used to validate mutation-specific selectivity of clinically relevant KRAS inhibitors
- 3D spheroid screening revealed enhanced KRAS dependencies, complementing the 2D screening for more comprehensive drug profiling
- Integrating mechanistic validation through isogenic lines and pathway analysis confirmed panel observations and provided deeper insight into selectivity
- Identification of MRTX1133 G12D/G13D cross-activity exemplifies the power of this approach to resolved nuanced selectivity patterns
- The panel delivers, clear, mutation-specific activity profiles for RAS pathway inhibitors and can be applied to diverse compound classes